REMARKS

Applicants acknowledge the Examiner's objection to the drawings under 37 C.F.R. 1.85(a). Applicants have amended the drawings to place them in proper form. Applicants have submitted, replacements for Figs. 8-11, and 13, and amended the remainder of the figures to be in compliance with the draftsman's requirements. Applicant's are still seeking to obtain additional replacement figures to further aid in the prosecution of this case. Applicants note with appreciation the Examiner's acknowledgement of the embedded hyperlinks, and as a result, Applicants have amended the Specification to remove reference to embedded hyperlinks contained within the text. Applicants' have canceled Claim 60, but do not disclaim the subject matter contained therein.

The Title of the invention has been objected to as lacking description. Applicants assert that the Title of the current invention is fully descriptive of the claimed invention, however, in view of the Examiner's suggestion the Applicants have amended the Title to read "GENETIC ENGINEERING OF PLANT CELLS TO PROVIDE ENHANCED EXPRESSION OF MULTIPLE FOREIGN GENES IN TRANSGENIC PLASTIDS UTILITIZING A SINGLE TRANSFORMATION EVENT."

The description of the Abstract has been objected to as to lacking description, and as result the Applicants submit herewith a new Abstract which is clearly indicative of the invention to which the claims are directed.

Turning to the objection of Claims 2-4, 6-7, 9, 15 and 60, the Applicants have amended the claims to include proper articles, correct spelling and fix minor typographical details. Applicant has canceled Claim 9 without disclaimer of the subject matter contained therein. Support for the Applicants use of the term "stoichiometric" is found on page 6, line 7; and page 26, lines 7-11. No new matter has been added.

The Applicants have attended to the rejections contained under 35 U.S.C. § 112, second paragraph to correct informalities within the claims.

Claim Rejections – 35 U.S.C § 112 Rejections

Claims 1-4, 6-7, 9, 15-20 and 60 are rejected under 35 U.S.C. § 112, first paragraph as lacking enablement. Applicants respectfully submit that the claims as amended, in view of the Specification, and in further light of the prior art which was known at the time the application was filed, provides complete enablement for a transformation vector, which is capable of introducing multiple genes into a target plastid genome by a single integration event. Applicants further submit that the Specification, in view of the knowledge in this field enables one skilled in the art to create a vector comprising any operon. Applicants submit that ,at the time the application was filed, there were a multitude of operons, which are well known in the art as a controllable unit of transcription consisting of a number of structural genes transcribed together and containing at least three distinctive regions; the promoter, spacer regions and coding sequences

An operon is well understood in the art to relate to a cluster of genes, the products of which all serve a particular function, wherein the expression of the genes in an operon is coordinated and regulated together. It is also well-known that one promoter drives an operon. As each gene in the operon has its own initiation codon and there must be the translation of the first gene in the gene cluster before there is translation of the second gene in the gene cluster. Each coding sequence should have its own ribosome binding site for initiation of translation by the ribosomes. A polycistron is the result of transcription of a multi-gene operon. Such operons included, but are not limited to, the lac operon, the his operon, the lac I operon, the mer operon and the Cry2Aa2 operon. Therefore, the Applicants have provided claims for a multi-gene operon, which is functional to co-

express multiple enzymes in the plastids. In other words, the Applicants do not claim all multi-gene operons, but rather claim multi-gene operons, which are functional to co-express multiple heterologous proteins within the plastids.

The Examiner's attention is invited to page 19, lines 6-16, wherein the Applicants have specifically defined types of operon which can be used in the Applicants' vectors. In light of the clear definition of the operons to be utilized in the application, Applicants submit that there is clear guidance for plastid transformation vectors comprising the operons as set out in the Applicants' Specification.

The prevailing standard for determining whether the Specification meets the enablement requirement is whether the experimentation needed to practice the invention is "undue" As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable coalition to discover the claim, then the enablement requirement is satisfied. *In re Wands* 8 USPQ2d 1400 (Fed. Cir. 1988).

The Applicants have incorporated by reference a universal vector capable of transforming the plastid genome of different plant species (page 20, lines 14-18). A universal vector construct integrates at least two genes into the spacer region between the chloroplast transfer RNA genes coding for isoleucine and alanine within the inverted repeat (IR) region of the chloroplast genome by homologous recombination. The integration into these transcribed spacer regions allow the genes to be inserted without interfering with the function of other native genes'. (Specification page 20, lines 28-30; page 21 lines 1-2).

For the Examiner's convenience we include below non-limited extracts, which illustrate that the Applicants were in possession of a universal chloroplast vector capable to be targeted to the chloroplast genomes of higher plants.

The invention provides universal chloroplast integration and expression vectors which are competent to stably transform and integrate genes of interest into chloroplast genome of multiple species of plants. Transformed plants and their progeny are provided. Monocotyledonous and dicotylendonous plants are transformed which have never been transformed heretofore.

It has been discovered contrary to the conventional belief, that the chloroplast (ct) genome of plants contains spacer regions with highly conserved nucleotide sequences. The highly conserved nature of the nucleotide sequences of these spacer regions of the chloroplast genome makes such spacer regions, it has been discovered, ideal for the construction of vectors to transform chloroplasts of widely varying species of plant, this without the necessity of constructing individual vectors for different plants or individual crop species, which would require first a determination of the DNA sequence of each of the chloroplast genomes.

The Universal Vector. The invention has several useful embodiments. The invention provides a universal integration and expression vector hereinafter referred to as "UV" and its use for the expression of at least one phenotype in a variety of different plants.

The integration expression universal vector of the invention comprises an expression cassette (further described below) which comprises the necessary genetic elements to transiently or preferably stably transform the plastids e.g. chloroplast genome of a target plant cell with a foreign (heterologous) DNA coding for a molecule of interest, like a phenotype to be expressed by the plant or a non-plant high value molecule, like a biologically active peptide (or polypeptide). The universal vector is constructed with a transcriptionally active region of a chloroplast genome that is highly conserved in a broad range of chloroplast genomes of higher plants. Preferably that region is the spacer 2 region; the intergenic spacer region between the t-RNA^{Ile} and the tRNA^{Ala} region. Such region is often referred to herein as a "spacer" region because in the chloroplast genome it is intergenic between several genes in the rRNA operon which is transcribed by one promoter. When built into the universal vector such region is generally referred to herein as a "border" or preferably as a "flanking sequence" or "flanking sequences". This is because in the universal vector, the operably joined genetic elements for transforming stably the plastid of the target plant are flanked on each side by a sequence i.e. a fragment of the spacer region. The flanking sequences in the vector and the spacer sequences in the chloroplast genome have sufficient homology to each other to

> undergo homologous recombination. The universal vector is inserted into the spacer of a transcriptionally active region in the chloroplast genome. Generally, the spacer region is positioned in the inverted repeat region of the chloroplast genome. The rest of the construct, i.e. other than the flanking sequences and the expression cassette, is generally referred to herein as the "vector" which comprises bacterial sequences, like the plasmid cloning vectors pUC, pBR322, pGEM or pBlueScript.

At the time this application was filed, it was well-known in the art that there were at least sixty transcriptionally-active spacer regions within higher plant chloroplast genomes. (Sugita, M., Sugiura. M., Regulation of Gene Expression in Chloroplasts of Higher Plants, Plant Mol. Biol., 32: 315-326, 1996). Specifically, Sugita et al. reported sixty transcriptionally-active spacer regions referred to as transcription units, as can be seen in Table II of the article. . There are a number of conserved intergenic spacer regions, among various plant species. Applicant respectfully submits that because the transcriptionally active spacer regions were known, a universal vector could be used in any of the spacer regions contained within a higher plant chloroplast genome. Sugita et al., provides one skilled in the art the location of the intergenic spacer regions in the plastid genome. Therefore, one skilled in the art could use the method taught in the Applicant's specification to insert a universal vector within these spacer regions. In fact, the potato and tomato chloroplast genomes have been transformed using tobacco chloroplast DNA flanking sequences from the Applicant's universal vector (Nature Biotech, Vol. 19, 870-875 (2001), Ruff et al.; and Plant Journal, Vol. 19, 209-216 (1999), Sidirov et al.). Sidirov et al. and Ruff et al., support the Applicant's fact that a universal vector can be used to insert genes into a variety of plants. Specifically, these references utilize the teachings of the Applicant to transform a plant other than tobacco. Therefore, transformation of multiple plant species is well known and understood by those skilled in the art.

Heifetz et al. (2000, Biochimie 82: 655-666) teaches that complete plastid genomes have

been sequenced from a variety of plant and algal species to provide a wealth of information regarding conservation of reading frames and regulatory sequences. Plastid-encoded messages have also been found to be regulated post-transcriptionally over an unusually broad range. Taken together, these factors illustrate that heterologous genes or operons can be inserted into the plastid genome in a site-specific manner and can be expressed at levels ranging from low to extremely high, or alternatively, the coding sequences of endogenous plastid genes can be mutated in a directed fashion by virtue of the high efficiency of plastid homologous recombination. This is particularly true in light of the publication of the complete DNA sequence and genomic maps of at least fourteen different plant species. The following complete chloroplast genome sequences were already available in Genbank at the time of filing of this application:

Marchantia polymorpha 121,024 kbp

Nicotiana tabacum 155,844 kbp

Oryza sativa 134,525 kbp

Epifagus virginiana 70,028 kbp

Pinus thunbergii 119,707 kbp

Zea mays 140,387 kbp

Arbidopsis thaliana 154,478 kbp

Triticum aestivum 134,540

Euglena gracilis 143,172 kbp

Cyanophora paradoxa 135,599 kbp

Odontella sinensis 119,704 kbp

Porphyra purpurea 191,028 kbp

Chlorella vulgatis 150,613 kbp

Mesostigma viride 118,360 kbp

Thus, one could, if so inclined, simply search appropriate spacer regions of the various plastid genomes. In addition, numerous incomplete chloroplast genome sequences are also available in the Genbank with more being added all the time.

The Applicants invite the Examiner's attention to Example 18 from the "Revised Interim Written Description Guidelines Training Materials" (copy enclosed), where it is pointed out that "one skilled in the art would recognize that Applicant was in possession of all the various expression methods necessary to practice the claimed invention."

Example 18, a method for producing heterologous proteins, correlates well with the Applicants' claims relating to plastid expression constructs, which provide for enhanced expression of several foreign genes through a single transformation event. In Example 18 the "*Neurospora crassa* mitochondria gene expression is essential to the function/operation of the claimed invention." Similarly, chloroplast expression of a multi-gene operon which is functional to co-express multiple enzymes in the plastids provides a functional limitation of the Applicants' claims. (Claim 1). Example 18 is drawn to an allowed genus despite the fact that there is only a single embodiment reduced to practice. In Example 18, the expression of B0-galactosidasem, specifically, provided enablement for a genus claim. The mitochondria only expressed one protein with the help of one integration vector.

Applicants' invention uses the universal vector to transform tobacco plastid with multi-gene operons with a muti-gene that is expressed as a fully functional Bt toxin. In other words, Applicants' claims are drawn to a defined genus, by illustrating an embodiment that is representative of the genus based on the expression of an operon through a single transformation event. Applying the same reasoning of Example 18 to the Applicants' claims as amended, any of a variety of operons which

the Applicants have described in detail on page 19, lines 6-16 and throughout the Specification, can be inserted into a tobacco plastid via the universal vector and subsequently tested for expression with the use of a selectable marker.

The Examiner's attention is invited to Fig. 14-18 which show a variety of transformation vectors capable of integrating multi-gene operons. Of note, Fig. 18 provides an example of species specific vectors for alfalfa, soybean, potato and tomato which offer examples of plants whose DNA sequences were not known at the time of the filing of this application. These examples teach one skilled in the art to create a species-specific vector without detailed knowledge of the DNA sequence of the chloroplast genome to be transformed. Applicants respectfully submit that Kanno et al, 1993, Curr. Genet. 23:166-174 cited in the current Office Action, is irrelevant in light of the knowledge relating to spacer regions, and in further view of the Applicants' own universal vector.

Claims 1-4, 6-7, 9, 15-20 and 60 have been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter in such a way as to reasonably convey to one skilled in the art the inventors had possession of the claimed invention. Applicants respectfully submit that at the time the application was filed, Applicants did not claim a specific piece of nucleic acid, but rather Applicants claimed a vector capable of incorporating an operon that is functional in a plastid genome. The Examiner's attention is once again invited to page 19, lines 6-16, wherein the Applicants refer to the structural features of the multi-gene operons. The Examiner is further asked to consider page 12 of this Response, which defines the functions and role of operons. In view of the foregoing, Applicants respectfully submit that the claims as amended are fully enabled under 35 U.S.C. 112.

Response to § 112, Second Paragraph Rejections

Applicants note with appreciation the Examiner's helpful comments concerning § 112, second paragraph issues, and as a result of these of suggestions, the Applicants have amended the claims accordingly. All of the elements incorporated into the claims are fully supported by the Specification, and as a result no new matter has been added. Specifically, support for the phrase "a heterologous DNA fragment", now contained in Claim 1, is fully supported by Applicants' Specification on page 8, lines 16-18. Applicants further submit that "the spacer sequence" is well understood and defined in the art owing to such publication as Publication No. WO 99/10513 and Kota et al. (1999). A "spacer region" is well understood in the art to be the region between the functional genes of a plastid genome. The art has recognized that the sequence flanking functional genes are know in the art as spacer regions. (See U.S. Patent Application No. 09/079,640).

Applicants respectfully submit as a result of the amendments, the rejections under 35 U.S.C. § 112, second paragraph are now obviated.

Response to § 102 Rejections

Claims 1 and 60 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Blowers et al. (WO 99/05265). Applicants respectfully submit that Blowers et al. fails to provide an enabling disclosure for a vector capable of integrating a multi-gene operon into the plastid genome. Specifically, the Examiner's attention is invited to page 57, lines 7-10 of Blowers et al., wherein it is stated:

> DNA gel blot analysis of these pSCO3 transformants **should** reveal that the *glpB-hph-aadA* expression cassette has integrated into the chloroplast chromosome at its targeted site (as was observed for the NT1 transformants).

Applicants respectfully submit that the foregoing construct was merely a prophetic example that showed no tangible proof that the entire operon was integrated and expressed in the chloroplast. In fact, Blowers et al. acknowledged that DNA gel bot analysis had yet to be performed due to insufficient plant material for *hph-aad* constructs, and as a result there was no guarantee that the expression cassettes containing *hph-aadA* had been integrated into the chlorplast chromosome at the expected site. (Blowers et al., page 53, lines 4-7). Applicants further submit that Example 2 of Blowers et al. merely confirms the expression of *hph* and not the expression of a stoichimetric ratio of *hph* and *aad.* (page 49, lines 20-24 to page 50, lines 1-13, and page 51, lines 20-24). Consequently, Blowers et al. and the result obtained in both Examples 2 and 3, does not indicate where, if, and whether the genes of the respective constructs were expressed in a stoichimetric ratio.

Furthermore, Professor Ana M. Bailey, co-inventor of the Blowers et al patent, was a Visiting Professor in Professor Henry Daniell's laboratory and an abstract was submitted at the 10th International Association for Plant Tissue Culture & Biotechnology meeting entitled "Expression of glpA/b Operon in transgenic chloroplasts to degrade glyphosate" by Drs. Amit Dhingra, Ana M. Bailey and Henry Daniell. However, in spite of repeated efforts by experienced investigators this operon was non-functional, and the abstract was withdrawn by the corresponding author and no presentation was made at the IAPTC & B meeting in June 2003. Till today, this project has not been successful. Declaration by the investigators is included for the Examiners consideration

As a result, Applicants respectfully submit that Blowers et al. fails to provide an enabling disclosure for the insertion of a multi-gene operon wherein the multiple genes are expressed in transgenic chloroplasts.

Response to §103 Rejections

Claims 1-3, 6-7, 9, 15-20 and 60 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kota et al. (1999, Proc. Natl. Acad. Sci. USA 96:1840-1845) in view of Daniell et al. (1994, NATO ASI Series, Vol H 86, Biochemical and Cellular Mechanism of Stress Tolerance in Plants, Cherry, ed. Springer-Verlag Berlin, pages 589-604).

Daniell et al. speculated that cry operons may be expressed in chloroplasts. However, this would have never been possible based on the chloroplast vectors illustrated in their publication. Chloroplast integration vector described does not even contain chloroplast DNA flanking sequences for strable integration of transgenes into the chloroplast genome via homologous recombination. The T1 and T2 terminators described are bacterial terminators; when introduced into the chloroplast genomes, they would have terminated transcription of transgenes and thereby eliminated any possibility for multi-gene engineering. In contrast, current invention describes chloroplast 3' UTRs that are poor transcription terminators but stabilize transgene transcripts. Thus, the chloroplast vectors described by Daniell et al. is inoperable in chloroplasts.

As a starting point, the Examiner's attention is invited to a recent U.S. Court of Appeals case, which reviewed and reiterated fundamental principles relating to obviousness rejections. *In re Lee* articulated that the factual inquiry as to whether references may be combined must be both thorough and searching. *In re Lee*, 61 USPQ2d 1430, 1433 (CA FC 2002). *In re Lee* further reiterates the well established principle that common knowledge and common sense, even if assumed to derive from an agency's expertise, do not substitute for authority when the law requires authority. Id., at 1435. In view of these principles the Examiner's attention is invited to the following passage of *In re Lee*:

> The factual inquiry whether to combine references must be thorough and searching. It must be based on objective evidence of record. This precedent has been reinforced in a myriad of decisions, and cannot be dispensed with. See, e.g., Brown & Williamson Tobacco Corp. v. Philip Morris Inc., 229 F.3d 1120, 1124-25, 56 USPQ2d 1456, 1459 (Fed. Cir. 2000) ("a showing of a suggestion, teaching, or motivation to combine the prior art references is an `essential component of an obviousness holding"') (quoting C.R. Bard, Inc., v. M3 Systems, Inc., 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232(Fed. Cir. 1998)); In re Dembiczak, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617(Fed. Cir. 1999) ("Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references."); In re Dance, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637(Fed. Cir. 1998) (there must be some motivation, suggestion, or teaching of the desirability of making the specific combination that was made by the applicant); In re Fine, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600(Fed. Cir. 1988) ("`teachings of references can be combined only if there is some suggestion or incentive to do so."') (emphasis in original) (quoting ACS Hosp. Sys., Inc. v. Montefiore Hosp., 732 F.2d 1572, 1577, 221 USPQ 929, 933(Fed. Cir. 1984)).
>
> The need for specificity pervades this authority. See, e.g., In re Kotzab, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1317(Fed. Cir. 2000) ("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed"); In re Rouffet, 149 F.3d 1350, 1359, 47 USPQ2d 1453, 1459(Fed. Cir. 1998) ("even when the level of skill in the art is high, the Board must identify specifically the principle, known to one of ordinary skill, that suggests the claimed combination.
>
> In other words, the Board must explain the reasons one of ordinary skill in the art would have been motivated to select the references and to combine them to render the claimed invention obvious."); In re Fritch, 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783(Fed. Cir. 1992) (the examiner can satisfy the burden of showing obviousness of the combination "only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references").

In view of this well established principle, Applicants submit that the current Office Action

fails to provide evidence to suggest it was possible to express a prokaryotic Bt operon as described in Daniell et al., in the vectors disclosed by Kota et al. As the Examiner has already frankly acknowledged, Kota et al. does not disclose vectors comprising a chaperonin coding sequence in the expression cassette or a Cry2Aa2 operon, and as a result, Kota et al. clearly failed to show the introduction of multiple genes in a single transformation of event. Kota et al. simply makes a prophetic statement that the entire Cry2Aa2 operon should be expressed in chloroplast, simply because chloroplast can express and process a polycistron. Kota et al. offers no showing of multiple genes expressed by a single promoter in a chloroplast.

Several such positive predictions have been made because chloroplast contain several native operons. However, several unique requirements should be fulfilled in order to achieve expression of a heterologous operon in transgenic chloroplasts. First, a large fragment of foreign DNA should be integrated. Prior to this invention, a maximum of ONLY two foreign genes have been integrated into plastid genomes. There was great uncertainty about the maximal size for integration of foreign gees. Second, after successful integration, there was great uncertainty whether a heterologous operon could be transcribed by a chloroplast promoter because other forein promoters are non-functional within plastids. Third, after successful transcription, there was great uncertainty whether heterologous intergenic spacer regions be recognized by chloroplast ribonucleases and foreign transcripts should be processed accurately for translation by native enzymes. Unlike viruses, chloroplasts are not known to make polyproteins. If such polyproteins were made from a foreign operon, then unique proteases should be present within plastids to process polyproteins at unique cleavage sites. Fourth, after successful processing, proper ribosome binding sites should be present that would facilitate binding of chloroplast ribosomes and initiate translation. Chloroplast genes contain such ribosome binding sites (GGAGG) placed exactly 5 nucleotides upstream of the start

codon. Any variation of this distance or nucleotide composition negatively affects the efficiency of translation. Sixth, in addition to the ribosome binding cite, it was uncertain that foreign transcripts would be unstable in the absence of chloroplast 3' UTRs (untranslated regions). None of the genes in heterolohous multi-gene operon contained 3' UTRs). None of the enzymes or proteins involved in this process have been characterized and they are expected to be nulear encoded and therefore, their recognition of foreign nucleotide or amino acid sequences is highly questionable. Finally, after translation, the heterologous proteins should be present in proper ratios to assemble the cubidal crystals (one chaperone molecule for one CRY protein) and either one should not be degraded more than the other by chloroplast proteases. Finally, formed cuboidal crystals in transgenic chloroplasts should not destabilize the chloroplast thylakoid membranes and negatively impact photosynthesis or growth of transgenic plants. Thus, accomplishing expression of foreign operons is a much greater challenge than prior simplistic speculations in the literature solely based on the presence of native operon in plastids. That is why, the first report of expression of multiple genes in a single transformation was featured on the COVER of Nature Biotechnology (January 2001, copy enclosed) with an explanatory cover story.

In fact, evidence points to a contrary conclusion, because the processing and/or cofactors necessary for the processing of polycistrons via the chloroplast had not yet been characterized when Kota et al. was published. Therefore, at the time Kota et al. was published, there was no evidence that multiple foreign gene transcripts would be properly processed and translated when expressed from a homologous promoter.

The Official Action spectulates that the current claims are obvious owing to the fact that Daniell et al. makes a suggestion to express an entire operon in tobacco chloroplast. (Page 597, paragraph 3). This prophetic suggestion provides no evidence on this record as to the ability to

express a multiple gene operon in chloroplast. Rather, the paragraph states that it is desirable to express an entire operon and that it **should result in the production of stable crystalline insecticidal protein**. Daniell et al. further states that the operon will be inserted and will be examined, however, they offer no evidence, which would substantiate the likelihood of expression of a bacteria operon via the chloroplast genome, owing to the fact that at that time there was an inadequate understanding of the processing of polycistrons within plastids. Up to the date of the Applicants disclosure, all foreign genes which had been engineered via the plastid genome, had been driven by individual promoters and three prime regulatory sequences. At the time of filing this Application, it was not known whether a three prime terminator and related regulatory sequences were necessary for the expression of individual genes of foreign operons. (see attached article on the role of 3' UTRs in multi-gene engineering in transgenic chloroplasts, Ruiz et al., Plant Physiology, July 2003 copy enclosed).

It was further believed that the processing of polycistrons within chloroplast was regulated by several environmental factors such as light. In fact, several studies have demonstrated that psbA5'UTR confers light dependent translation not only to the psbA gene but also to other heterologous proteins. (Zergies, W. 2000) Translation in Chloroplast. Biochimie, 82, 583-601; Eibl et al. (1999), *In vivo* analysis of plastid psbA, rbcl. and rpl32 UTR elements by chloroplast transformation: Tobacco plastid gene expression is controlled by modulation of transcript levels and translation efficiency. *Plant J.* 19, 333-345: Staub et al. (1993) Accumulation of D1 polypeptide in tobacco plastid is regulated via the untranslated region of the psbA mrnA. *EMBOJ.* 12, 601-606: Staub et al. (1994), Translation of psbA mrnA is regulated by light via the untranslated region in tobacco plastid. *Plant J.* 6, 547-553.)

Furthermore, neither Kota et al. nor Daniell et al. suggest ribosome binding sites or

untranslated regions upstream of the bacteria genes could function within the plastids or processing sequences in spacer regions. Given these vast uncertainties surrounding the production and processing of multiple foreign genes in the plastid, there was no reasonable likelihood of success.

Furthermore, neither reference teaches or suggests that a chaperonin present in the bacterial cell could function within the chloroplast to help fold the foreign protein or interfere with folding of other chloroplast proteins. It is well understood in the art that *E. coli* does not form mature polypeptides, because *E. coli* does not form disulphide bonds in the cytoplasm. Consequently, to make a mature polypeptide, the polypeptide must be target in the periplasm to form disulfide bonds. Finally, neither of these references suggest that it was possible to create a cuboidal crystal within chloroplast to duplicate the functions of a bacterium during sporulation or duplicate the bioremediation pathway within plastids. Until the Applicants' invention, there was no reasonable predictability that the enzymes, and pathways of the operon could be expressed in a coordinated manner.

The prior art had even suggested the potential for unforeseen deliterous effects when there is a high level of expression of multiple foreign proteins within chloroplast. It has been repeatedly speculated that high-level expression of foreign proteins may be deleterious to planr growth and reproduction and yet current invention showed no such effect in spite of huper-expression of multiple transgenes. In fact, as is well understood in the art the pH and oxidation state of the chloroplast differ substantially from bacterial cells. These physiological differences were thought to inhibit or prevent functions of bacterial proteins and/or enzymes within chloroplast. The Applicants, however, have shown otherwise.

Claims 1, 2 and 60 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Turkec (1999, Turk, J. Field Crops 4:85-90) in view of Baumann et al. (1988, J. Bacteriol 170:2045-

2050). Applicants respectfully traverse this rejection and submit that in view of the claim amendments and further view of the remarks set forth above and below, the rejection is now obviated.

Applicants respectfully submit that Turkec et al. is incompatible with the Applicants' disclosure. Turkec et al. is related to the transformation of *Chlamydomonas reinhardtii*, which is an organism considered both a protozoon and an algae (Bold and Wynne, Introduction to the Algae, 2d. ed. page 84). Specifically, Turkec et al. related to algae plastid transformation. From there, the Official Action assumes without evidence on this record, that because of relative DNA sequence homology between the chloroplast genome of higher plants and that of *Chlamydomonas reinhardtii* there would necessarily be similar integration into the chloroplast genome of higher plants. This assumption ignores the fact that the Applicants have used spacer regions which are homologous across all higher plant species, but which are not homologous with the chloroplast genomes of *Chlamydomonas reinhardtii*.

The Examiner's attention is invited to the following passage from Turkec et al.:

> The present work demonstrated that foreign DNA (mosquitocidal binary toxin genes) has been introduced into the chloroplast genome of *Chlamydomonas reinhardtii*. However, expression level of these genes has been found lower than expected. Complex pattern of integrated fragments have been found lower than expected. Complex pattern of integrated fragments have been found in transformed colonies, probably due to multiple recombination events (Goldschmidt-Clermont (1991) and/or the homology of the introduced DNA to the chloroplast genome of *Chlamydomonas reinhardtii* (Boynton, et al., 1990). **Therefore, further experiments are needed to achieve stable transformation of these genes in *Chlamydomonas reinhardtii***. For this reasons, northern, western hybridization and bioassay analyses will also be carried out to show to express binary toxin genes in these positive colonies. Our results showed that chloroplast genome is not easily manipulated as mentioned by Suzuki, et al., (1997).

Turkec et al. expressed that further experiments were needed to achieve stable transformation of muliple genes within the *Chlamydomonas reinhardtii* chloroplast genome, and also indicated that the chloroplast genome is not easily manipulated and that substantially more efforts were needed to achieve the aim of introducing multiple genes into chloroplast. In view of this admission and in further view of the difficulties involved in expression of multiple genes in chloroplasts, Applicants submit that Turkec et al. makes no suggestion to transform the chloroplast genome of higher plants with a multi-gene operon. Applicants further submit that Turkec et al. teaches the use of **multiple promoters** to drive the individual genes, rather than a single promoter which drives the Applicants entire operon. As a result, Turkec et al. can not achieve the transcription of two proteins in the same stoichiometric amount, as the Applicants have demonstrated on page 26 of the Specification compare with Turkec et al., page 86 in materials and methods. Turkec et al. invention is distinctly different because they use more than one promoter contrary to the current invention. Finally, Chlamydomonas contains a single chloroplast in a single cell. In contrast, plant cells contain multiple chloroplasts with about 10,000 copies of plastid genomes per plant cell. Therefore, stably integrating 10,000 copies of transgenes requires an entirely different approach than integrating a few copies of transgenes into Chlamydomonas cells.

Moreover, Turkec et al., fails to teach the translation of binary toxin genes, and therefor provides no motivation to one skilled in the art to express an operon in a plant plastid Rather, Turkec et al merely shows that there is complex integration patterns of transgenes. This teaching is not surprising because it had not been possible to translate a multitude of foreign genes in the past, even though integration and transcription were reported by several investigators. After almost twenty years of the first stable transformation of Chlamydomonas chloroplasts, investigators are now able to express very low levels of transgenes, after extensive codon modifications. For example,

GFP, which has been repeatedly expressed in higher plant chloroplasts without any modification was finally expressed in Chlamydomonas chloroplasts after extensive codon modification. Please see enclosed article by Mayfield et al (2003) that appeared in PNAS in January 2003 that gives several examples of codon modification of transgenes to achieve very low levels of translation. In contrast, inventors of this application show accumulation of up to 47% of total protein from cry2A operon, without any codon optimization. Thus one skilled in the art would, upon reading Turkec et al, in light of the art at the time of the Applicant's invention would not believe it was possible to use the teachings of Turkec et al. in combination with Baumann et al.

In view of the foregoing, Applicants respectfully submit that Claims 1, 2 and 60 are clearly patentable over Turkec in view of Baumann et al.

Claims 3 and 4 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Turkec in view of Baumann et al. as applied to Claims 1, 2 and 60 and in further view of Crickmore et al. Applicants respectfully traverse this assertion and submit that for the reasons set forth above, that Claims 3 and 4 are clearly patentable over Turkec et al. in view of Baumann et al. and Crickmore et al.

Claims 1 and 60 have been rejected under 35 U.S.C. §103(a) as being unpatentable over McBride et al. (U.S. 5,545,817 A, 1996). Applicants respectfully submit that McBride et al. fails to teach a plastid transformation vector that introduces multiple genes in an operon. Rather McBride et al. merely speculates as to the use of a series of individual genes. (see challenges pointed out above in detail). McBride et al. merely provides an expression construct having two unrelated genes, and as a result the insertion taught by McBride does not produce a polycistronic mRNA strand. Once again, the Examiner's attention is invited to *Milestones in Chloroplast Generic Engineering: An Environmentally Friendly Era in Biotechnology, Trends in Plant Science, Vol. 2, No. 2, February*

2002, wherein the use of second gene in the production of the Bt toxin resulted in a substantial increase of toxin (up to 47% of the total syimprotein). McBride et al. failed to accomplish such a result. Furthermore, McBride et al show individual genes driven by individual promoters and 3' UTRs. No consideration was given for processing of spacer regions, multiple genes driven by a single promoter or expression of foreign proteins in stoichiometric amounts.

The Examiner has directed the Applicants' attention to column 2, lines 56-63, of McBride et al. which states, without evidence or scientific data, that the plastid construct of interest may contain a number of consecutive encoding regions to be expressed as an operon. Applicants respectfully submit that nowhere in McBride et al. is there an example or illustration, which demonstrates a multi-gene operon expressed using a single promoter in an individual transformation event. At the very most, McBride et al. offers an invitation to attempt the expression of polycistrons in a single transformation event. The Examiner's attention is invited to the case of *Amgen Inc. v. Chugai Pharmaceutical Co., Ltd.*, 18 USPQ2d 1016-1023 (Fed. Cir. 1991), wherein the Court addressed the often mis-applied "obvious to try" standard involving a biotechnology patent. Of note, the Federal Circuit concluded that the probing strategy employed by Amgen while potentially obvious to try failed to offer any reasonable expectation of success because the strategy had not been successfully used before. Id. at 1022. Similarly, McBride et al., while hypothesizing potential for introducing a number of consecutive coding regions into the plastid genome, fail to offer any detailed teaching, examples or illustrations that would provide one skilled in the art with the suggestion that there would be a reasonable expectation of success. This is especially true in light of the problems the Applicants overcome to express polycistrons in a single transformation event. These hurdles as were detailed throughout the Specification and this response, clearly illustrate the number of problems to express a polycistron in a single transformation event.

In view of the foregoing, Applicants respectfully submit that the rejection of Claims 1 and 60 under McBride et al. are now obviated.

Claims 1 and 60 have been rejected under 35 U.S.C. § 103 as being unpatentable over Maliga et al. Maliga et al. shows insertion of a promoterless GUS gene downstream from an indulgenceness rbcL wherein the indulgenceness rbcL has a weak terminator creating a potential for a read through. Maliga et al. has merely introduced a **single transgene** which exhibits read through to a native gene. This **has been previously documented in the literature. most importantly, maliga et al. presents evidence that the expression of promoterless gus is highly variable compared to rbcl. such an invention would be of no value in expressing multi-subunit proteins or enzymes in a pathway**. In essence, Maliga et al. have shown that a transgene may take advantage of an inefficient chloroplast terminator for expressing a single foreign gene but with no suggestion of a multi-gene operon. Furthermore, nothing in Maliga et al. suggest that there were ribosome binding sites upstream of individual cistrons contained within the vector or processing of heterologous spacer regions. The Applicants, however, have successfully used the bacteria operon with bacteria ribosome binding sites and spacer regions, which were recognized by a chloroplast so that reading was initiated, stopped, then restarted at the next cistron to make multiple individual polypeptides.

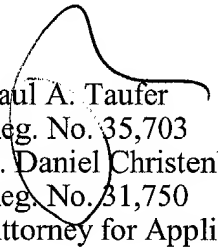
One skilled in the art, after reading the disclosure in this application, will understand that the Applicants have provided a means by which multiple genes can be transcribed and produced in stoichimetric ratios. Maliga et al., however, does not achieve stoichimetric amounts of transcript, owing to the fact that read through is a random event and thus, coordinated expression of multiple genes is not possible. The Examiner's attention is invited to page 6, lines 6-10, Fig. 3 lane 5, page 22, lines 19 to page 23, line 25 and page 26, lines 7 and 8, wherein it is noted that the proteins should be produced in equal amounts, i.e. the same stoichimetric ratio.

Double Patenting Rejection

In as much as Claims 1-4, 6-7, 9, 15-20 and 60 have only been provisionally rejected under the judicially created doctrine of obviousness-type double patenting, Applicants respectfully submit that the provisional rejection can be held in abeyance.

In view of the foregoing, Applicants respectfully submit the claims as amended are now in condition for allowance, which action is respectfully requested.

Respectfully submitted,



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